## INTERACTION ANALYSES OF LIVE, ATTENUATED TETRAVALENT DENGUE VACCINES - EVIDENCE OF INTERFERENCE AND COMPLEMENTATION

**Anderson KB, Gibbons RV, Putnak R, Eckels K, Edelman R and Sun W**

Live, multivalent vaccines may exhibit interference in human subjects. To identify possible interactions and optimize doses for tetravalent dengue vaccines, four dengue (DEN) vaccine viruses were administered to volunteers as: 1) full dose monovalent vaccines (n=31) and 2) 16 tetravalent vaccine formulations derived from a factorial design with all combinations of full and low dose dengue vaccines (n=61). For the factorial analyses, increasing the dose of DEN-1, DEN-3, or DEN-4 resulted in significantly increased geometric mean titers (GMTs) to that serotype. For DEN-2, the GMT was higher with a low dose of DEN-2. DEN-1 and DEN-3 exhibited mutual interference at full doses, while a full dose of DEN-3 boosted GMTs to DEN-4. The greatest reactogenicity was observed when DEN-1 was at full dose and all others were low; the addition of other serotypes at full doses decreased the reactogenicity of the formulation. The lowest reactogenicity was observed when all serotypes were at full dose. Data comparing monovalent vaccines to full dose tetravalent formulations are pending. These findings were tested against new dose formulations with vaccine viruses of earlier passage history. The model correctly predicted that increasing the dose of DEN-1 was associated with increased GMTs to DEN-1, 2, and 3 (direct relationship). An inverse relationship was observed with an increased dose of DEN-1 and GMTs to DEN-4. This conflicted with model predictions but may be attributable to the difference in passage history. The model correctly predicted that reactogenicity would decrease with a higher dose of DEN-1. These data suggest that viral interaction occurs in tetravalent dengue vaccine formulations, with respect to both antibody titers and reactogenicity. Both interference and enhancement were observed in antibody responses. Interestingly, increasing the dose of non-DEN-1 serotypes decreased adverse effects of the vaccine. Factorial designs may be useful in designing optimal vaccine formulations by predicting the effects of changes in dose.

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## METHODOLOGY OF THE PROSPECTIVE STUDY OF DENGUE VIRUS TRANSMISSION AND DISEASE IN PRIMARY SCHOOL AND VILLAGE CHILDREN IN KAMPHAENG PHET, THAILAND

**Mammen MP Jr, Jones JW, Pimgate C, Koenraadt S, Zhang C, Thammapalo S, Srikiatkhachorn A, Libraty DH, Green S, Scott T, Getis A, Morrison A, Endy TP, Ennis FA and Rothman AL**

An improved understanding of the correlations between the host, viral, and environmental factors and dengue disease severity will contribute to dengue virus (DV) vaccine development. The goal of the study is to identify factors that have the strongest influence in determining the early events in acute DV infections, and the eventual clinical manifestations of disease. An

equally important goal is to characterize protective immune responses (e.g. $CD4^+$ and $CD8^+$ T-cell responses, neutralizing antibody responses) as it has been found that low levels of pre-existing neutralizing antibodies to a subject's own infecting virus isolate do not necessarily protect from symptomatic DV infection. We aim to prospectively identify host-specific factors (e.g. pre-existing memory T and B cell responses to DV, HLA genetic polymorphisms, viral burden and replication in the host), virus-specific factors (e.g. DV serotype, serotype infection sequence), and environmental factors (e.g. mosquito population patterns, mosquito viral burden) for asymptomatic and symptomatic secondary DV infections, particularly severe infections (DHF/DSS). Multi-year investigations are planned to study the year-to-year variations in the incidence and prevalence of circulating serotypes.

The study employs school- and village-based components. Approximately 2000 children from 11 primary schools are actively followed for acute symptomatic DV infections. A 1-dilution screening neutralization assay is performed bracketing each dengue season to identify asymptomatic DV infections. Selected school-derived positive and negative dengue RT-PCR results from acute specimens serve to establish index cases for the initiation of cluster investigations within specific villages, the houses of which have been pre-mapped by GIS and pre-characterized as to number of children and their ages. Upon initiation of a cluster investigation, 10-25 child contacts (6mo-15 yrs of age) are identified within a 100-meter radius of the index case and are evaluated by questionnaire serially for 15 days for DV infection with blood samples taken on days 0 and 15 regardless of illness. Mosquitoes are collected within the 100-meter radius and are tested utilizing dengue RT-PCR. Mosquito spraying is further conducted to halt local DV transmission.

The methodology of this multi-collaborative study will be described to include quality assurance (QA) systems employed to have this study serve as a bridging study to the future testing of dengue vaccines in phase 2 and phase 3 clinical trials.

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## THE MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS SEROTYPE 2 AND 3 CIRCULATING IN THAILAND FROM 1974 TO 2001

**Zhang C, Mammen MP Jr, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P and Holmes EC**

Dengue represents a major public health problem in Thailand, with all four viral serotypes co-circulating. This provides an optimal setting to investigate the molecular epidemiological history and evolutionary trends of these co-circulating dengue virus (DENV) serotypes in a highly endemic country; to characterize intra-serotypic variations of DENV in a locality; to determine the evolutionary forces shaping viral genetic diversity; to determine whether the changing prevalence of DENV could be attributed to instances of adaptive evolution in the viral genome. Furthermore, it permits the investigations of whether molecular determinants of the envelope (E) gene of DENV correlate with disease severity. To this end, we undertook a large-scale molecular epidemiological analysis of 173 E gene sequences of DENV representing